# PREDOMINANCE AMONG THE MEMBERS OF THE BACILLUS COLI GROUP IN ARTIFICIALLY STORED WATER *

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## THE EFFECT OF STORAGE

Up to recent years, the presence of typical Bacillus coli in a water supply has been considered as an index to recent pollution. The exponent of this view, Houston, believes that the class of organisms which ferment lactose and produce indol in peptone broth is indicative of recent pollution. It has been shown however by Peckham (1897) that the indol reaction is highly variable. MacConkey (1905) has divided the bacillus coli group into four large sub-groups according to their ability to ferment saccharose and dulcite, a classification which has received almost universal approval. According to MacConkey, and later Jackson (1908), all organisms fermenting lactose may be divided into the four following groups:

| Group | Saccharose | Dulcite |
|---|---|---|
| Bacillus communior | + | + |
| Bacillus communis | — | + |
| Bacillus aerogenes | + | — |
| Bacillus acidi lactici | — | — |

The sign + means gas production.

By far the most complete work on the practical application of this classification to the problem of recency and remoteness of pollution has been carried on by Major W. W. Clemesha.[1] In his work on the highly polluted water supplies of India, he has found that the groups fermenting dulcite, Bacillus communior and Bacillus communis, are sensitive organisms, found only in numbers where pollution is recent, and that the groups negative to dulcite, Bacillus aerogenes and Bacillus acidi lactici, are highly resistant forms, which occur in numbers when the pollution has been remote or the water stored for a long time.

In Houston's classification of the bacillus coli group into "Typical and Atypical," the groups fermenting dulcite, B. communior and B.

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1. Bacteriology of the Surface Waters in the Tropics, 1912.

communis, which are considered by Clemesha as indicating recent pollution, do not fall into the class of true colon bacilli. This has led to a long discussion. According to Clemesha, "true and atypical coli are purely laboratory classifications; they are not in the slightest degree based on a study of the natural characteristics of fecal organisms, and in estimating the age of a pollution they are of no value." In reviewing Clemesha's work, one must realize that conditions under which he worked were very different from those confronting workers in England and America, and that the hot Indian climate, populated by a people whose diet is very different from ours, offers a different problem so far as the purity of a water supply is concerned.

Stimulated by the discussion between Clemesha and Houston, workers in this laboratory undertook to study the effect of storage on predominance among the various members of the bacillus coli group, according to the MacConkey classification. The routine analyses were carried out by P. Astrofsky and I. Cohen, whom I wish to thank for their co-operation.

In October, 1913, ten one-gram portions of feces from five of the laboratory assistants were emulsified in 1,000 c.c. of tap water in the common one-liter bottles of the reagent type. Each bottle was plugged with cotton to exclude further contamination, while at the same time allowing a free circulation of air. Five of these bottles were placed in a covered box in a dark corner of the laboratory, while the comparative five were placed in a position where light could easily play upon them. The temperature, at all times, was approximately 20-21 C. In February, 1914, another series of five bottles from five other students was similarly stored in the light. At the beginning of each series of experiments, the emulsion of each bottle was well shaken and 1 c.c. and its dilutions were plated on litmus lactose agar and incubated at 37 C. for twenty-four hours, after which the total and bacillus coli counts were made. From the plates representing each bottle, twenty-five to thirty colonies at random were fished onto agar slants, from which inoculations were made into lactose peptone bile as a presumptive test for membership in the bacillus coli group, and into dulcite and saccharose broth to determine their classification according to the MacConkey scheme. This same procedure was followed out each week until the bottles failed to yield any more members of the group. During the course of the experiments, some 4,000 cultures were isolated and studied according to this method. Early in the work it was found that individual labeling was impossible and so elastic bands were used to keep the culture and its corresponding fermentative tubes together, the tubes from each bottle being kept in a separate basket. It may be said that all media were made according to the Standard Methods as proposed by the American Public Health Association. At first, in determining gas production, the regular fermentation tubes were used, but very soon inverted vials were substituted, both for convenience and for ease of cleansing. The lactose peptone bile used as a presumptive test for membership in the bacillus coli group was sterilized in test tubes 5 x 5/8

inches with inverted vials 2 x ¼ inches. The dulcite used for differentiating the cultures according to the MacConkey scheme, as a result of its cost and scarcity, was sterilized in homeopathic vials 2 x ⅜ inches with the inverted vials ⅛ x 1 inch. The latter were made in the laboratory by cutting ⅛ inch glass tubing into lengths of 1¼ inches and sealing the end with a blast flame. The saccharose was sterilized in the same manner as the bile. The fermentative tubes were incubated at 37 C. for forty-eight hours, as the maximal gas production always occurred by, or before, that time. Results are expressed as positive or negative gas production, as the author believes that percentages of gas production are of no value, since the reaction, especially of bile, determines the amount of gas produced.

Table 1 gives the initial frequency of various members of the bacillus coli group in the fresh feces, classified according to the MacConkey scheme.

TABLE 1

FREQUENCY OF MEMBERS OF THE BACILLUS COLI GROUP IN FRESH FECES

| Number of Sample of Human Feces | Bacillus communior | Bacillus communis | Bacillus aerogenes | Bacillus acidi lactici |
|---|---|---|---|---|
| 1 | 4.3 | 78.5 | 0 | 17.2 |
| 2 | 0 | 100.0 | 0 | 0 |
| 3 | 0 | 100.0 | 0 | 0 |
| 4 | 32.0 | 20.0 | 0 | 48.0 |
| 5 | 47.0 | 35.3 | 0 | 17.7 |
| 6 | 0 | 5.0 | 15.0 | 80.0 |
| 7 | 0 | 0 | 6.2 | 93.8 |
| 8 | 0 | 3.7 | 3.7 | 92.6 |
| 9 | 0 | 4.1 | 0 | 95.9 |
| 10 | 0 | 0 | 0 | 100.0 |
| 11 | 7.7 | 0 | 7.7 | 84.6 |
| Summary | 8.3 | 31.6 | 3.0 | 57.2 |

Total count of bacteria was 175 organisms.

These results may be compared with the results of other workers.

| | Bacillus communior | Bacillus communis | Bacillus aerogenes | Bacillus acidi lactici |
|---|---|---|---|---|
| MacConkey | 23.0 | 37.0 | 15.0 | 25.0 |
| Clemesha | 6.8 | 17.4 | 22.2 | 53.2 |
| Winslow and Walker | 28.0 | 60.0 | 4.0 | 8.0 |
| Graham Smith | 43.0 | 17.0 | 11.0 | 29.0 |
| Browne | 8.3 | 31.6 | 3.0 | 57.2 |

It may be well to state that in this work Samples 1-5 inclusive were examined in October, Sample 6 in December, and Samples 7-11 inclusive in February. The results obtained seem to indicate that there is not only a great individual variation in the flora of the feces, but a seasonal variation as well. Clemesha has pointed out that there are epidemics of certain organisms in the feces. If such is the case, it is highly important to know the conditions governing the presence of the various forms during the various periods.

TABLE 2

THE EFFECT OF STORAGE IN DIFFUSE LIGHT UPON THE PRESENCE OF THE VARIOUS MEMBERS OF THE BACILLUS COLI GROUP (1ST SERIES)

| Bacillus Coli Group | Number of Days Stored in the Light | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 8 | 13 | 20 | 31 | 38 | 47 | 56 | 69 | 73 |
| B. communior | 16.7 | 31.9 | 37.3 | 26.3 | 43.5 | 20.7 | 38.5 | 24.9 | 27.9 | 23.7 |
| B. communis | 66.9 | 41.7 | 25.0 | 35.6 | 31.6 | 27.6 | 34.1 | 39.8 | 22.6 | 42.8 |
| B. aerogenes | 0.0 | 1.0 | 21.0 | 21.6 | 3.5 | 14.5 | 2.3 | 4.3 | 8.9 | 3.1 |
| B. acidi lactici | 16.4 | 25.4 | 16.7 | 16.5 | 21.4 | 37.2 | 25.1 | 31.0 | 40.6 | 30.4 |

The percentages represent a summary of the counts made in five samples.

In the study of Table 2, certain results seem to stand out: The percentage of frequency of Bacillus communior increases in all five samples during storage, a result out of accord with Clemesha's work, in which he maintains that Bacillus communior is a weak organism and never multiplies on storage. Bacillus communis, on the other hand, shows a tendency to weaken but is by no means absent at the end of seventy-three days. Bacillus aerogenes does not seem to be present in very large numbers in any of the samples. The fourth member of the group, Bacillus acidi lactici, shows a tendency to increase during storage. If, however, we examine the total percentage of positive dulcite fermentation in each bottle at the beginning and at the end of each experiment, we find that the results are nearly alike.

| | Percentage of Dulcite Fermentation | |
|---|---|---|
| | Initial Frequency | Final Frequency |
| Sample 1 | 84 | 89 |
| Sample 2 | 100 | 94 |
| Sample 3 | 100 | 100 |
| Sample 4 | 52 | 64 |
| Sample 5 | 82 | 35 |

If, as Clemesha states, fermentation of dulcite represents recent pollution, then these results do not accord with his, for here the percentage of frequency in some cases increases.

In the series of experiments in which the bottles were kept in the dark, an attempt was made to imitate the conditions below the surface of a body of water, and thus see whether light has any effect upon the presence or absence of the various members of the group. As has been stated, these bottles were inoculated with a gram of fresh feces in the same manner as the bottles stored in the light because it had been shown by Clemesha that laboratory cultures behave differently from organisms obtained directly from the intestinal tract.

TABLE 3

THE EFFECT OF STORAGE IN THE DARK UPON THE PRESENCE OF THE VARIOUS MEMBERS OF THE BACILLUS COLI GROUP (1ST SERIES)

| Bacillus Coli Group | Number of Days Stored in the Dark | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 13 | 20 | 31 | 38 | 47 | 56 | 69 | 73 |
| B. communior | 16.7 | 34.5 | 56.3 | 59.9 | 14.9 | 26.5 | 41.5 | 25.2 | 38.0 |
| B. communis | 66.9 | 27.5 | 22.8 | 26.1 | 22.3 | 15.3 | 42.7 | 57.7 | 49.5 |
| B. aerogenes | 0.0 | 14.9 | 7.7 | 2.0 | 36.1 | 25.1 | 5.1 | 3.7 | 12.5 |
| B. acidi lactici | 16.4 | 23.1 | 13.2 | 13.0 | 26.7 | 33.1 | 10.7 | 27.4 | 0.0 |

The percertages represent a summary of the counts made in five samples.

The results in Table 3 seem to substantiate those in Table 2. Bacillus communior shows an initial frequency of 16.7 percent, and a final frequency of 38 percent at the end of seventy-three days, an increase of over 100 percent. Bacillus communis shows the same gradual decrease as before, and the other two forms reach their highest point midway and decrease toward the seventy-third day. It is interesting that the final positive fermentation of dulcite is either more than or equal to the initial fermentation of dulcite.

| | Percentage of Dulcite Fermentation | |
|---|---|---|
| | Initial Frequency | Final Frequency |
| Sample 6 | 84 | 100 |
| Sample 7 | 100 | 100 |
| Sample 8 | 100 | 100 |
| Sample 9 | 52 | 75 |
| Sample 10 | 82 | 63 |

Tables 2 and 3 indicate that the presence of light has little or no effect upon the presence of the various members of the bacillus coli group.

In February, the second series of bottles was inoculated and stored in the room, as the previous experiments had shown that light had little or no effect upon the presence of members of the bacillus coli group. This series was conducted along the same lines exactly as the December series and so the two are directly comparable.

TABLE 4

THE EFFECT OF STORAGE IN DIFFUSE LIGHT UPON THE FREQUENCY OF THE VARIOUS MEMBERS OF THE BACILLUS COLI GROUP (2ND SERIES)

| Bacillus Coli Group | Number of Days Stored in the Light | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 28 | 35 | 42 |
| B. communior | 1.5 | 12.3 | 2.0 | 23.3 | 24.8 | 21.0 |
| B. communis | 1.5 | 5.7 | 8.8 | 26.3 | 15.4 | 32.2 |
| B. aerogenes | 3.5 | 0.8 | 4.6 | 0.0 | 2.5 | 2.0 |
| B. acidi lactici | 93.5 | 81.2 | 84.6 | 50.4 | 57.3 | 44.8 |

The percentages represent a summary of the counts made in five samples.

Table 4 shows that the results obtained in the second series of bottles substantiate those obtained in the first series: Bacilli communior and communis, which, according to Clemesha, are supposed to be the least resistant forms, increase in frequency many fold. Bacillus aerogenes plays the same inert part. Bacillus acidi lactici, according to Clemesha the more resistant form, decreases in frequency.

### THE EFFECT OF ENRICHMENT AND STORAGE

During the second series of experiments (in February), a comparative test was made to see whether enrichment in lactose peptone bile had any effect upon predominance among the members of the bacillus coli group. The procedure was the same as that described above except that, at the time when the direct litmus lactose agar plates were made from the fecal emulsions, lactose peptone bile tubes were inoculated with 1 c.c. of the emulsion and incubated for twenty-four hours at 37.5 C. Litmus lactose agar plates were made from the bile tubes and cultures isolated as in the direct plating. The procedure may be illustrated as follows:

| | Not Enriched | Enriched |
|---|---|---|
| Feb. 17 | Various dilutions from each bottle plated on litmus lactose agar plates, and incubated at 37.5 C. twenty-four hours. | One c.c. from each bottle enriched in lactose peptone bile and incubated at 37.5 C. twenty-four hours. |
| Feb. 18 | One hundred and twenty-five colonies fished from five bottles onto agar slants, and incubated at 37.5 C. twenty-four hours. | The enriched cultures plated on litmus lactose agar plates and incubated at 37.5 C. twenty-four hours. |
| Feb. 19 | | One hundred and twenty-five colonies fished from five bottles onto agar slants and incubated at 37.5 C. twenty-four hours. |
| Feb. 21 | Inoculation from each streak made into dulcite and saccharose broth, and lactose peptone bile; incubated at 37.5 C. seventy-two hours. | Each streak inoculated into dulcite and saccharose broth and lactose peptone bile and incubated at 37.5 C. seventy-two hours. |
| Feb. 24 | Recorded gas. | |

Other conditions not mentioned here are exactly as stated in the first part of the paper.

TABLE 5

THE EFFECT OF ENRICHMENT IN LACTOSE PEPTONE BILE UPON PREDOMINANCE AMONG THE MEMBERS OF THE BACILLUS COLI GROUP ISOLATED FROM STORED WATERS

| Bacillus Coli Group | Number of Days Stored | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | 7 | | 13 | | 27 | | 34 | | 41 | |
| | a | b | a | b | a | b | a | b | a | b | a | b |
| B. communior..... | 1.5 | 1.6 | 12.3 | 8.9 | 2 | 4.6 | 23.3 | 24.1 | 24.8 | 17.3 | 21 | 15.9 |
| B. communis..... | 1.5 | 5 | 5.7 | 15.4 | 8.8 | 0.7 | 26.3 | 17.7 | 15.4 | 7.8 | 32.2 | 27.2 |
| B. acidi lactici..... | 93.5 | 90.6 | 81.2 | 72.3 | 84.6 | 91.6 | 50.4 | 57.2 | 57.3 | 72.4 | 44.8 | 56.0 |
| B. aerogenes..... | 3.5 | 2.5 | 0.8 | 3.2 | 4.6 | 3.1 | 0 | 0.8 | 2.5 | 2.3 | 2.0 | 0.7 |

The percentages represent a summary of the counts made in five samples.
a = not enriched in lactose peptone bile.
b = enriched in lactose peptone bile.

The results of these experiments, as shown in Table 5, corroborate those brought out earlier in the paper.

## CONCLUSIONS

A comparison of the feces taken during different periods indicates a differentiation in the flora of the bacillus coli group, as evidenced by the fermentation of dulcite and saccharose.

Bacillus communior, a dulcite-fermenting organism, which has been regarded as an index to recent contamination, not only holds its own, but increases in prevalency during storage.

Bacillus communis, the other dulcite-fermenting member of the bacillus coli group, also regarded as an index to recent contamination, altho it rarely ever shows an increase in prevalency during storage, nevertheless decreases very gradually and slowly.

The frequency of fermentation of dulcite by organisms isolated from fresh feces, and that by organisms from fecal emulsions stored seventy-three days, approximate each other very closely.

The presence of light or darkness does not seem to affect the ratio of the four groups.

Enrichment in lactose peptone bile does not seem to favor the growth of one member of the bacillus coli group over the others; for, in comparative tests, those strains which were enriched in lactose peptone bile appeared in the same percentage of frequency as those strains which were not enriched.